**Week 1 Immunology Cases:**

***Case 25: Severe Congenital Neutropenia (SCN)***

**Summary:** Neutrophils are phagocytes that play a role especially with infections with extracellular bacteria. Innate immune system. First cells to be recruited to sites of infection and inflammation. Short lived. After ingesting microbes and killing them they die and are broken down by macrophages and produce pus. Recognize targets by binding to invarient receptors such as FcGamma and TLR. Opsonized microorganisms bind to Fc receptors on neutrophil surface and promote their internalization and killing (reactive oxygen species, proteolytic enzymes, antimicrobial peptides in granules). Generated in bone marrow via hematopoietic stem cells which differentiate into myeloblasts then to promyeloblasts then to metamyelocytes (cell loses ability to divide). High rate of production in the bone marrow required to balance the rapid rate of neutrophil destruction/short half life. Decreased production of neutrophils either from BM suppression/malfunction or increase consumption/destruction.

SCN presents very early in life with recurrent often severe bacterial infections involving skin, umbilical stump, soft tissue, lungs, and deeps organs. Can have sepsis. High risk of invasive fungal infections. Marked neutropenia (often less than 200 cells per microliter). Monocyte and eosinophil counts normal or elevated. Serum Ig levels are increased. Characteristic findings of maturation arrest at promyelocyte or myelocyte stage in BM. Can be caused by a lot of things but most talked about is a problem with G-CSF receptor that normally causes myeloid cells to develop into neutrophils. Can be due to mutations in ELA2, anti apoptotic protein HCL2, p14 endosomal protein, TF GF independent, adaptor protein comple 3, Wiskott Aldrich syndrome gene. Chronic neutropenia.

**Case:** Umbilical cord stump infection. Fever. Lethargic and hypotonic, Rapid HR, Fast and shallow breathing. Distended abdomen that was warm to touch and red. Decreased neutrophil count. Positive staph culture. Bone marrow aspirate showed normal numbers of granulocyte progenitors but severe block in neutrophil differentiation at promyelocyte stage and almost complete lack of more mature forms (shows its not due to bone marrow suppression). Mutation in ELA2 gene for enzyme neutrophil elastase. Later diagnosis with acute myeloid leukemia monsomy chrom 7.

**Treatment:** vancomycin (antibiotic), recombinant human granulocyte colony stimulating factor (increased stimulation of early myeloid progenitors)

**Describe the pathogenesis of Severe Congenital Neutropenia (SCN).**

Block of myeloid differentiation in the bone marrow at the promyelocyte stage (maturation arrest), impacts more mature cells with normal levels of immature and progenitor cells

Selective decrease of circulating neutrophils, occurance of infections

Genetic mutations including genes for elastase 2, HAX1 anti apoptotic protein, p14 endosomal protein, TF GF independent, and subunit for adaptor protein complex

Response with treatment of recombinant human granulocyte colony stimulating factor (increased stimulation of early myeloid progenitors?)

**Explain how you differentiate between increased peripheral destruction of neutrophils and decreased production of neutrophils in the bone marrow as a cause of neutropenia.**

Increased peripheral destruction: ability to produce neutrophils in bone marrow should be normal, BM aspirate would show normal levels of myeloid cells in all stages of differentiation

Decreased production: reduction of myeloid cells in the BM either in all stages (leukemia) or more mature myeloid cells with normal amounts of immature and progenitor cells (SCN)

**Explain why neutrophil transfusions rarely used in the treatment of Severe Congenital Neutropenia (SCN).**

Rapid turnover of neutrophils so they would die within a day, risk of inflammatory reactions

**Explain why somatic mutations of G-CSF receptor cause an increased risk of leukemia.**

Cytokine binding promotes intracellular signaling leading to proliferation and differentiation, need negative regulation via internalization or proteasome degradation of cytokine receptors

Intracytoplasmic tail of G CSF receptor has a ubiquitination site, trunctation impedes ubiqutination and causes increased signaling and cellular hyeractivation

***Case 36: Rheumatoid arthritis: type IV hypersensitivity rxn***

**Summary:** Adaptive immune system incites inflammation and causes tissue damage. Rheumatic disease is inflammation and damage to the joints and can be caused by a subset of autoimmune diseases (SLE, Sjogren’s syndrome, scleroderma). Chronic. Characteristic by inflammation of synovium (thin lining of the joint). Inflammed synovium causes invasion and damage of cartilage followed by bone erosion and joint destruction. Autoreactive CD4 T cells become activated by self antigen presented by dendritic cells or activated by pro inflammatory cytokines produced by macrophages. Autoreactive effector T cells can then recognize Ags within the joint and maintain the inflammatory reaction. Can also help to potentiate autoreactive B cells leading the plasma cell differentiation and producing autoantibodies. Rheumatoid factor is IgM autoantibodies which are directed against Fc portion of IgG (not seen in all patients and can be in healthy people too). Abs against citrullinated proteins also commonly found (but some pts don’t present this). MHC allele mutation common, also for other immune system proteins.

**Case:** morning stiffness. Wrist and finger swelling (spongy indicates thickening of synovium). Rheomatoid factor in blood. Low hematocrit (slightly anemic). Slighly low WBC count. Very high sefimentation rate. Normal urinalysis. X ray showed erosions in several joints.

**Treatment:** monthly IV infusions of infliximab (monoclonal Ab against TNF alpha, risks include antibody production against it because its human mouse chimeric and may produce an anaphylactic rxn), adalimumab (anti TNF monoclonal antibody that is completely humanized so it doesn’t elicit and immune response), Etanercept (soluble form of TNF receptor, traps TNF in solution), Others: anakinra (antagonist of IL-1R, prevents integrin expression so leukocytes can’t leave through BV wall), tocilizumab (an anti-IL-6R antibody), Rituximab (delete peripheral B cells specifically one's w/ CD20--depletes levels of RF), abatacept (fusion protein of CTLA 4 and Fc portion of IgG, costimulatory molecule inhibitor).

**Explain that there are several autoimmune rheumatic diseases (i.e. diseases affecting the joints; Fig 42.1).**

**Explain that rheumatoid arthritis (RA) is associated with a Th1-mediated inflammatory reaction in joints, and that this includes the production of TNF**

Unknown trigger sets up initial inflammation in synovial membrane and attracts autoreactive lymphocytes and macrophages to the inflammed tissue, synovial membrane becomes thickened and increased growth of BVs, synovium infiltrated by autoreactive CD4 T cells which activate macrophages and result in the production of pro inflammatory cytokines such as TNF alpha, IL 6, IL 1, IL 17, IFN gamma, cytokines induce production of matrix metalloproteinases by fibroblasts contributes to tissue destruction, TNF family cytokine RANK ligand is expressed by T cells and fibroblasts in the joint and is the primary activator of bone destroying osteoclasts resulting in joint destruction. Also, B cells are activated by binding of CD40 to CD40L and B7.1 to CD28-->activated and secrete Ig's including RF into synovial fluid which activates complement.

TNF alpha and IL 1 enhance infiltration of leukocytes into joint space by upregulating the expression of integrin CD11:CD18 on leukocytes which promotes their binding to BV walls and emigration from the vessels.

**Describe the action of the several drugs available that interfere with TNF activities, including those that trap TNF in solution (e.g. Etanercept is a soluble form of the TNF receptor) and Infliximab (antibody against TNF).**

**Define “rheumatoid factor” and explain that its presence is not, by itself, diagnostic of RA, since several other diseases are associated with rheumatoid factor and it may also be present in persons with no obvious autoimmune disease.**

Other illnesses include hypergammaglobulinemia and chronic inflection

**Recognize that stiffness in the joints, first noticed after getting up from bed and lasting at least an hour, is a common early sign of RA.**

**Explain that like SLE, a number of criteria for diagnosis are needed to confirm RA. For RA, this includes simultaneous arthritis in several joints.**

Includes morning stiffness for at least 1 hr. before max improvement, arthritis of 3 or more joints simultaneously with swelling or fluid in joints, arthritis in hand joints with wrist swelling, symmetrical arthritis of same joint areas, rheumatoid nodules, serum rheumatoid factor, radiographic changes

**Explain that RA occurs less commonly in men than in women.**

**Explain that there are certain HLA-DR alleles that are more common in patients with RA than would be expected from their frequency in the general population, and that this suggests that certain specific peptides may be particularly arthritogenic.**

Certain alleles for MHC class II alleles for CD4 T helper cells during Ag presentation events

Mast cells needed for expression of arthritis in mice because they express the IgG receptor FcgammaRIII which they use to take up IgG:Ag complexes to cause immediate release of preformed INF alpha and Il 1

***Case 37: Systemic lupus erythematosus (SLE), type III hypersensitivity rxn***

**Summary:** Immune complexes produced when there is an Ab response to a soluble Ag which progress in size as the immune response progresses and triggers complement activation. C1 (q, r, s) binds soluble immune complexes and lead to activation of C4, 2 and 3. C3b associates with the Ag-containing complex. Complexes are cleared by binding C3b on immune complexes to CR1 on RBCs which carry the complexes to the liver and spleen. They are removed from RBCs through interaction with complement and FcGamma receptors on Kupffer cells (macrophages in the liver) and phagocytes. When Ag is repeatedly released there may be sustained formation of small complexes that can be trapped in blood vessels of renal glomeruli and synovial tissue of the joints. Excessive production of ICs' can overwhelm any residual RBC clearance mech and these soluble complexes tend to become lodged in the walls of small BV's in the kidney glomeruli (causing glomerulonephritis) and the synovial tissues (causing arthritis) of joints causing IC disease.

SLE is the most prevalent immune complex disease. Characterized by formation of antibodies against DNA (rich source of DNA via nuclei extrusion from erythroblasts in the bone marrow as they mature into RBCs). More common in women. Often have autoantibodies against multiple autoantigens. Small ICs are produced and primarily lodge in kidney (also synovial tissues in joints). Tissue injury mediated by activation of complement due to the presence of ICs.

Patients often produce autoantibodies against different components (ex: nucleosome or ribonucleiprotein particle which contain many different molecules) which have many separate epitopes. Any of the Abs can bind to the nucleosome and form an IC. The potentially autoreactive B cells probably exist in normal circulation, but aren’t activated because of T cell tolerance (T cells aren’t reactive against same autoantigen). SLE is probably caused by a failure of T cell tolerance. All the autoreactive B cells are probably actived by a single clone of autoreactive T cells specific for a peptide of one of the proteins in the complex. (B cell bind to complex component, internalizes and degrades it, and returns peptides to surface bound to MHC II which then stimulate Th cells, which in turn activate B cells)

Cytokine pathways also play a role including type 1 interferons alpha and beta that are secreted in response to triggering of Toll like receptors. Type 1 IFNs promote activation of autoreactive T cells and augment class switch and B cell Ab production. IRF5 is a TF used in IFN synthesis and is identified as a suspetibiltiy factor for SLE. Small percentage of patients on IFN alpha treatment develop lupus.

**Case:** 16 yr. old girl. Butterfly rash on face after sun exposure. Morning stiffness. Symmetrical swelling in fingers. Blood sample showed anti-nuclear antibodies against dsDNA. Normal platelet count, negative direct and indirect Coombs tests (establishes presence of autoimmune hemolytic anemia which occurs when there are Abs against RBCs). Negative test for anti-phospholipid. Normal urine sample (if glomerulonephritis it would have contained protein and RBCs). After a month had worsened morning stiffness, fever and chills at night, enlarged lymph nodes, weight loss, no rash, diffuse swelling of proximal joints in fingers and toes, low C3 levels in serum. High serum IgG levels (constant stimulation of B cells by autoantigens leads to increased number of plasma cells secreting Igs) \*NOTE: lymph node biopsy would show follicular hyperplasia in cortex and increased numbers of plasma cells in medulla

**Treatment:** hydroxychloroquine sulfate (antimalarial agent, good for skin lupus), avoid direct sunlight (UV light provokes SLE and evokes relapses), prednisone, naproxen (nonsteroidal anti inflammatory)

**Explain that soluble immune complexes that activate complement are initially bound by C1, leading to the production of the active forms of C4, C2 and C3, which can subsequently bind to any of the immune complex components (including C1); this results in C3b being associated with the antigen-containing complex.**

**Recognize that CR1 on red blood cells binds C3b on immune complexes, which allows the immune complexes to be carried by RBCs to the liver and spleen.**

**Recognize that macrophages in the liver and spleen remove IgG-containing immune complexes from RBC surfaces via binding of the Fc regions to Fc receptors on the phagocytes.**

**Explain that excessive production of immune complexes can overwhelm any residual RBC clearance mechanisms. These soluble complexes tend to become lodged in the walls of small blood vessels in the kidney glomeruli and the synovial tissues, and thereby cause “immune complex disease.”**

**Recognize that glomerulonephritis and arthritis are frequently seen in persons with SLE.**

**Recognize that subacute bacterial endocarditis, mixed essential cryoglobulinemia, and systemic lupus erythematosus (SLE) all represent examples of “immune complex disease.”**

Subacute bacterial endocardities has bacterial autoantigen and results in glomerulonephritis

Mixed essential cryoglobulinemia has autoantigens to rhematoid factor IgG complexes and causes systemic vasculitis

SLE has autoantigens to DNA, histones, ribosomes, snRNP, and scRNP and causing glomerulonephritis, vasculitis, arthritis

**Explain that SLE is a disease that is quite variable in its expression, and that diagnosis is often based on a patient showing several, but not necessarily all, clinical features among several criteria. Know that these features include a butterfly rash and production of anti-nuclear antibodies with specificity for double-stranded DNA.**

Butterfly rash on face that is evoked by exposure to the sun, symmetric morning stiffness, enlarged lymph nodes

**Explain that progressive forms of SLE are associated with low CH50 and depression in C3 concentration (complement is consumed), and that under such conditions corticosteroids are often required to control disease progression.**

Serum C3 and C4 are lowers by the large number of ICs binding C3 and C4, triggering their cleavage. Depletion of protein is proportional to severity of the disease.

**Recognize that SLE is far more common in women than men.**

**Explain that autoantibodies to several cellular components are found in SLE, but not necessarily all targets are seen in each patient. Antigen targets include ribonucleoproteins (fairly commonly), and more rarely phospholipid complexes, platelets, and RBCs.**

Most common is against dsDNA and ribonucleoproteins

**Recognize that SLE may be induced and exacerbated by excessive sunlight exposure.**

Unknown reasons

***Case 40: Multiple sclerosis (MS): type IV hypersensitivity***

**Summary:** T cells recognize self-peptide:MHC complexes and become activated, leading to local inflammation by activating macrophages leading to tissue damage. MS is a T cell mediated autoimmune disease. In mice, injection of myelin cause neurological symptoms similar to MS (called experimental autoimmune encephalomyelitis). Antigens in myelin that induce EAE include myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG). T cells specific for these antigens have been found in blood and CSF of MS patients. More common in women. Associated with HLA-DR2. Plaques show dissolution of myelin along with infilitrates of lymphocytes and macrophages particullarly along blood vessels (inflammation increases vascular permeability).

Unknown trigger provokes release of CNS antigens and presents them to lymphocytes in peripheral lymphoid organs resulting in expansion of autoreactive T cells and sets up initial focus of inflammation in brain, blood brain barrier becomes locally permeable to leukocytes and blood proteins, activated autoreactive TH1 cells specific for CNS Ag cross barrier via alpha4:beta1 integrin that binds to vascular adhesion molecules on surface of venule endothelium and migrates out of blood into the brain, and reencounter Ag on MHC class II microglial cells, T cells and macrophages secrete pro inflammatory cytokines such as IFN gamma and IL 17, exacerbation of inflammation and further influx of macrophages, dendritic cells, and B cells as well as complement proteins to the site in CNS, inflammatory rxn in the brain due to mast cell activation and histamine release, complement activation, Ab production against myelin antigens by autoreactive B cells, and cytokines, leads to demyelination of neurons and interferance with neuronal function.

**Case:** female in good health, loss of vision in 1 eye, eye movement was normal and had no pain, retina was normal, neurological exam normal, optic neuritis (inflammation of optic nerve), family history of MS (mother), MRI revealed multiple lesions in white matter of brain under cortex and around ventricles (IV gadolinium injection showed that this inflammation was probably recent), treatment with corticosteroids, 3 years later: developed weakness in mm. of L side of the face innervated by 7th cranial n., MRI showed new lesions in L middle cerebellar peduncle and pons, CSF has normal protein but high IgG content (due to B cells in CNS after blood brain barrier breakdown) and high lymphocytes, electrophoresis showed clonal expansion of restricted B cell populations in CNS, treatment with IFN beta, 3 years later: weakness in L hand and leg, slurred speech, nystagmus, ataxia, IFN stopped and put on high doses of cyclophosphamide and corticosteroids

**Treatment:** IV corticosteroids, cyclophosphamide (cytotoxic drug), both of these inhibit T cell proliferation and interfere with cytokine secretion to decrease inflammation and T cell activation, weekly intramuscular injections of interferon beta (unknown mechnaism), natalizumab (removed from market, targets alpha 4 integrin to block leukocyte movement from blood into sites of inflammation)

**Explain that T cells appear to be the primary inducers of inflammation found in the autoimmune disease, multiple sclerosis (MS).**

**Describe that in MS, the T cells respond to CNS antigens that are normally not detected by the immune system. However, once T cells are exposed to the CNS antigens and become activated, they home to the CNS cause disease.**

**Explain why IFN- treatment for MS is contraindicated, since it induces MHC class II expression.**

IFN gamma upregulates MHC II expression and enhances Ag presentation, also drives the differentiation of Th1 cells

**Describe that current treatment includes the use of cytotoxic and inflammatory drugs.**

**Explain that oligoclonal IgG is characteristic for the CSF in MS. This is because only a very few of the B cells that enter the CNS have activity for CNS antigens, and so a limited diversity of IgG molecules is seen.**

**What is the rationale behind feeding MBP to mice to prevent EAE?**

Feed mice MBP to prevent EAE because when proteins are eaten they are known not to elicit immune response. Ag specific mechanism in gut to suppress peripheral immune responses to Ag delivered by mouth (T cells lack co stimulatory signals, development of regulatory T cells that can actively suppress Ag specific responses by producing cytokines like IL 4, IL 10, TGF beta to inhibit development of TH1 responses.) Feeding MBP to humans has not worked.

**Will EAE be induced in CD28 knockout mice?**

EAE can’t be induced in mice lacking CD28 because it is the receptor on T cells for B7 co stimulatory molecules needed to the activation of naïve T cells (including T cells to recognize MBP). Knockout CTLA 4 in mice induces EAE more readily (receptor for B7 to deliver inhibitory signal to activated T cell)

***Case 42: Myasthenia gravis: type II hypersensitivity***

**Summary:** Anti IgG antibodies against ACh Receptors on cell surface at the NMJ (adaptive immune response). Abs against the receptor stimulate internalization of the receptor via endocytosis leading to its degradation and prevents mm. from responding to neuronal impulses. Abs also mediate complement mediated lysis of the m. endplate which damages mm. membrane. See fluctuating weakness that worsens w/ activity and improves w/ rest. Pregnant women w/ MG transfer disease to newborn b/c IgG is only Ig that crosses the placenta (proves that is is caused by an anti-IgG Ab). May also have autoantibodies for muscle specific kinase (MUSK), which is a tyr kinase involved in clustering Ach receptors. \*Defining characteristic is fluctuating weakness that worsens with activity and improves with rest (fewer Ach receptors mixed with physiological decrease in Ach after sustained physical activity leads to m. weakeness). Can have worsening symptoms after infection by exposing hidden Ags from behind a cell or tissue barrier, local inflammation may trigger MHC expression and costimulators on tissue cells to start and autoimmune response, infectious agents may bind to self proteins, pathogens binds to self protein and acts as carrier to allow anti-self response, molecular mimicry (production of cross reaction Abs or T cells), superantigen for polyclonal activation of autoreactive T cells

**Case:** diplopia (double vision) that sometimes improved simultaneously, ptosis of both eyelids, most often first seen in laryngeal / ocular muscles--eyelids are droopy (oculobulbar form, most common); Serum presents Ab against ACh receptor. After severe respiratory infection: ptosis very severe, poor speech, difficulty in breathing (decreased vital capacity) and in chewing and swallowing food. Weakness of facial mm. and tongue

Older pts. Have more generalized m. weakness and often autoantibodies against m. proteins as well

Chest x ray of young ppl w/ MG often show enlargement of thymus (association with thymomas, neoplastic ep cells in thymoma express selflike epitopes that resemble proteins like the ACh receptor, also decreased autoimmune regulator gene AIRE and smaller numbers of regulatory T cells)

**Treatment:** pyridostigmine (inhibitor of cholinesterase, side effect is diahrea because it binds to muscarinic receptors in the intestine and increases intestinal motility), treated with azathioprine (cytotoxic agent that is immunosuppressant), plasmapheresis (removal of plasma from whole blood) to treat a crisis, thymectomy may help improve systems if removed early

**Define the targets of autoimmune responses for the syndromes listed in Fig 40.1.**

Autoimmune hemolytic anemia: Rh blood group antigens, l antigen

Autoimmune thrombocytopenic purpura: Platelet integrin Gpllb:llla

Goodpasture’s syndrome: Noncollagenous domain of basement membrane collagen type IV

Pemphigus vulgaris: Epidermal cadherin

Graves’ disease: Thyroid stimulating hormone receptor

Myasthenia gravis: Acetylcholine receptor

Insulin-resistant diabetes: Insulin receptor (antagonist)

Hypoglycemia: Insulin receptor (agonist)

**Recognize that myasthenia gravis is characterized by the development of progressive weakness.**

**Recognize that signs and symptoms of myasthenia gravis may be transferred to a normal person by injecting IgG from a patient, and therefore infants can develop disease when the mother has myasthenia gravis because IgG is transferred across the placenta.**

Disease can last 1-2 weeks, within this time all the maternal IgG antibodies against the receptor are adsorbed from the baby’s blood.

**Describe the treatment of myasthenia gravis as using a cholinesterase inhibitor (this lengthens the half-life of acetylcholine after its release from motor neurons) and recognize that more aggressive treatment uses cytotoxic agents.**

Azathioprine is an immunosuppressive agent, converted in liver to merceptopurine which inhibits DNA synthesis and inhibits growth of rapidly dividing cells such as B and T cells. This is non specific so patients are susceptible to infections. Can also be associated with lymphoma development with prolonged use.

**Explain that, in younger patients, there is an association between enlargement and abnormalities of the thymus and the development of myasthenia gravis, and that in this population, thymectomy can lead to rapid improvement in symptoms.**

**Recognize features of eye and eyelid movement that are associated with oculobulbar myasthenia gravis (see description and Fig. 40.3).**

When gazing R: R eye moves and L eye stays straight; when gazing L: L eye moves and R eye stays straight.

***Case 49: Acute Systemic Anaphylaxis: type I hypersensitivity***

**Summary:** Inappropriate immune responses to innocuous foreign Ag resulting in a hypersensitivity reaction via activation of sensitized CT mast cells. Can be from any protein allergen including venom, latex, and penicillin (acts as hapten). Requires latent period for sensitization (on first exposure allergen specific IgE Abs/T cells are generated which bind to FceRI receptors on mast cell surface). Second exposure leads to allergic reaction (by cross linking of IgE bound to FceRI on mast cells and basophils that leads to degranulation and release or mediators such as histamine). Histamine leads to increased permeability of BVs which can cause hypotension. Type 1 IgE mediated hypersensitivity reaction (allergen captured by B cells through IgM and processed so peptides are presented to MHC II to TCRs on Th2 cells, activation of specific Th2 cells leads to production of IL4/Il13 and induces prodction of IgE). Rapid onset. Involvement of at least 2 organs systems (skin, respiratory, GI, CV, CNS). Effects on circulation and respirtory are most dangerous (localized swelling of upper airway can cause suffocation). GI symptoms include nausea, vomiting, diarrhea. Symptoms can occur single or in combo. Skin can be a route of allergen sensitization. Hoarseness due to angioedema of vocal cords. Wheezing due to forced expiration of air through bronchi that have become constricted due to activation of mast cells (histamine and leukotrients that caused the smooth m. of the bronchial tubes to constrict). Increased incidence of peanut allergy due to increased topical use of peanut oil based creams to treat dry skin.

**Case:** Swollen lips after eating peanut butter cookies (first exposure). Month later ate cookies and vomited, became hoarse, difficulty breathing, wheezing, swollen face, lethargic and LOC. Hypotension, rapid HR and RR. Labored breathing. Blood test for histamine and tryptase to see if they were released from activated mast cells.

**Treatment:** epinephrine (acts on beta 2 adrenergic receptors surrounding the BVs and bronchi, increased constriction of BVs smooth m. to stop vascular leakage and raise BP, relaxes bronchi to make it easier to breath), anti inflammatory corticosteroid methylprednisolone, albuterol (beta2 adrenergic agent), can deplete IgE by admin of humanized mouse anti-human IgE Ab because it results in depletion of mast cell bound IgE.

**Describe how allergens sensitize persons and lead to the development of allergies.**

**Describe the clinical result of mast cell activity at different sites (Fig 32.3), and explain why there is a drop in blood pressure and airway constriction in systemic anaphylaxis.**

**Explain why allergen exposure in the gut or skin may result in allergens entering the circulation, and that this may cause effects at distant organs (e.g. asthma).**

**Explain the action of epinephrine (adrenaline) in causing increased constriction of the blood vessels’ smooth muscle, but relaxation of the airway’s smooth muscle, so it causes an increase in blood pressure and airflow in a person with anaphylaxis. Recognize its availability in the Epi-Pen format.**

**Describe how intravenous introduction of an allergen can lead to multiple areas of mast cell degranulation and this can cause systemic anaphylaxis.**

**Describe oral and respiratory routes of allergen exposure as being less likely to cause severe anaphylaxis than are the intramuscular, i.v. and subcutaneous routes.**

**Recognize that medications, and especially penicillin, cause the majority of anaphylactic deaths in the USA. Also, explain that horse serum used in antitoxins was a major sensitizer, but is less commonly used nowadays because of effective vaccination and human-based antitoxins.**

**Explain that, following massive mast cell degranulation, a patient is largely unresponsive to allergens due to depletion of mast cell granules (i.e. tachyphylaxis), and that recovery takes 3-4 days.**

**Be able to explain the use and procedure of the radioallergosorbent test (RAST).**

Radioallergosorbent test (RAST) is used to determine which substances a person is allergic to (also remove peas, can cross react with peanuts and could elict a reaction). Skin test should be done days later because of tachyphylaxis which asts 72-96 hrs. after allergic rxn (depletion of mast cell granules and failure of BVs to respond to mediators)

***Case 50: Allergic Asthma: hypersensitivty reaction type I***

**Summary:** Most common chronic inflammatory disorder of the airways, reversible inflammation and obstruction of small airways. Genetic predisposition is called atopy. Wheezing and coughing are main symptoms (due to forced expiration of air that have been narrowed due to smooth m. constriction).More common than acute systemic anaphylaxis. Allergic reaction becomes worse with each subsequent exposure to allergen (worse symptoms and more T cells and Abs). Also have hypersensitivity during exercise, cold air, viral infection and pollutants (generalized hyperresponsive, degree can be measured by determining threshold dose of inhaled methacholine that produces a 20% reduction in airway flow, irritabilty correlated with eosinophils and IgE levels). Severe uncontrolled asthma can lead to airway remodeling

First exposure to allergen causes Th2 cells to release IL4 which drives B cells to produce IgE in response to the Ag. Allergen specific IgE binds to mast cells via the FceR1. Second exposure causes cross linking to bound IgE molecules and relase of mast cells granule contents (histamine) to increase blood flow and vascular permeability – early phase. Within 12 hrs. a late phase rxn occurs when arachidonic acid metabolism in mast cells generates prostglandins and leukotrients which further increase blood flow and vascular permeability. Cysteinyl leukotrienes can bind to rec 1 and lead to bronchial smooth m. constriction and m. cell proliferation, plasma leakage, hypersecretion of mucus, and eosinophil migration. Cytokines (IL3, 4, 5, TNF alpha) are produced by mast cells and Th cells to prolong the reaction. Causes an influx of monocytes, T cells, and eosinophils to site. Eosinophils in particular make products that cause tissue damage and mucus production. Invarient NKT cells (thymus derived, expression markers of both T cells and NK cells) are elevated also produce cytokines (Th1 type IFN gamma, IL-4, 13, 2, and TNF alpha). Also increased secretion of IL 4, 5, 9, and 13 by Th2. Neutrophil-dominated asthma may be a distinct phenotype and is associated with increased IL 17 expression.

**Case:** Persistant wheezing that didn’t respond to bronchodilator (beta2 adrenergic agonist albuterol), first wheezing attack when exposed to dog and happened every time after that, seasonal allergies, cold weather and exercise brings on coughing and wheezing (sometime responded to albuterol), night time cough, hospital admission (treated with broonchodilators and IV steroids), maxillary sinusitis with episodes associated with green nasal discharge and asthma exacerbation, intermittent sneezing, nasal itching and congestion, eczema that cleared up, family history of asthma and allergic rhinitis, reduced peak expiratory flow rate, forced expiratory volume reduced, chest Xray showed hyperinflation of lungs (air traped in lung), high eosinophil count, high serum IgE, RAST showed IgE antibodies for allergens, normal IgG, A, and M levels, nasal fluid had eosinophils.

**Treatment:** corticosteroids (inhibit transcription of allergic and pro inflamm cytokines, also activate transcription of anti inflamm ones leading to decreased numbers of mast cells, eosinophils and T cells in bronchial mucosa), leukotriene agonists to inhibit synthesis to cause bronchodilation and anti inflammation), anti IgE therapy with a humanized monoclonal Ab (complexes with free IgE and presents binding onto Fce receptor on mast cells to decreased circulating free IgE and downregulation of receptor on cell surfaces), beta 2 agonists (bind to rec. on bronchial smooth m. cells and relax them, good for immediate phase treatment), minimize exposure to allergens, immunotherapy by injecting antigens to try to desensitize.

Steroids given to combate late phase reaction

**Describe the steps involved in development of allergy. These follow a primary exposure and sensitization step in which Th2 cells release IL-4, a cytokine that is needed for B cells to switch to produce IgE.**

Th2 production results in production of Il4 and IL 13 that induce IgE production, also IL5 for eosinophil maturation.

**Recognize IgE is produced by plasma cells and becomes attached to the Fc receptors on mast cells and basophils.**

**Recognize that cross-linking of IgE molecules on mast cell or basophil surfaces leads to release of fast-acting mediators found in granules (e.g. histamine).**

**Describe the steps involved in developing a wheal and flare response in a skin test with allergen.**

Tests for IgE mediated hypersensitivity to and allergen. Histamine released by mast cells in skin which increases BV permeability and content leakage into tissues (wheal) and dilation of BVs around the area to produce the red flare. Rxn can be inhibited by antagonist of histamine type 1 receptor.

**Explain the steps involved in the initial asthmatic response to allergen and the response of the airway to histamine.**

**Recognize that a secondary asthmatic response follows the production of mediators such as leukotrienes and prostaglandins, and that these are produced during the inflammatory response occurring in asthma.**

**Recognize that an influx of eosinophils is a hallmark of an ongoing inflammatory reaction induced by mast cell degranulation.**

**Define “atopy” and “atopic,” and recognize that families can show predisposition to developing allergies.**

**Explain that a Th2 activity is associated with the development of atopy, and that IL-5 produced by Th2 cells is needed for eosinophil maturation.**

**Describe the process of immunotherapy used to desensitize allergic patients, including that it involves repeated subcutaneous injection of fairly high doses of allergen to promote switching to a Th1 response (Question #8 on page 205).**

IL12 production leads to Th1 production, Th1 produces IFN g which prevents isotype switiching to IgE. IgG competes with IgE for antigen and binds the allergen to inhibit mast cell activation and B cell activation by binding to FceR and giving inhibitory signals. Also no furthur boosting of IgE production because IL 4 and 13 aren’t secreted.

**Explain that allergens typically sensitize persons who are exposed to miniscule quantities of allergens presented via the mucosal route (Question #9 on page 205).**

Th2 response when allergens are presented in very small amounts via the mucosal route (B7.2 on APCs favors Th2 development) leading to IL4 production and IgE. Large doses subQly results in Ag uptake by lymph nodes and favors Th1 production which inhibits IgE production.

Sensitivity to NSAIDs which can induce wheezing because of COX inhibition which inhibits prostaglandin synthesis from AA. Leads to more AA to the leukotrient pathway to produce bronchial smooth m. constriction and cell proliferation, plasma leakage, mucus hypersecretion, and eosinophil migration

***Case 51: Atopic Dermatitis: hypersensitivty reaction type I***

**Summary:** Common pruritic (itching) inflammatory skin disease often associated with family history of allergy. Often starts in infancy and improves or resolves by age 5 (although 15% of cases persist into adulthood). Develop other indications of atopy (genetic predisposition to develop allergies). Hallmark is skin barrier dysfunction resulting in dry itchy skin leading to scratching (injury) and subsequent access to Ags resulting in sensitization and allergice skin inflammation. Scratching also causes tissue damage that stimulates keratinocytes to secrete cytokines and chemokines (il1, 6, CXCL8, GMCSF, TNFa). IL1 and TNFa induce expression of adhesion molecules (e selectin ICAM, VCAM) on endothelial cells to attract lymphocytes, macrphages, and eosinophils into the skin. These secrete cytokines and inflammatory mediators to activate keratinocytes more and causes cutaneous inflammation. Patients have increased transepidermal water loss (indicates disrupted barrier function). Filaggrin is a gene found in the epidermal differentiation complex on chrom 1 that is mutated in about 20% of patients. The protein is needed during corneocyte differentiation and is degraded to hydrophobic aa’s that play a role in skin hydration.

Damage to the skin barrier allows allergens to penetrate into the subepidermal layer and interact with langerhans and dendritic cells. Keratinocytes produce cytokines (IL1, 6, TGF b and TSLP) which leads to maturation and migration of APCs to the draining lymph node where they present Ags to naïve T cells and skew differentiation toward Th2 cells. Activated Ag specific T cells proliferate and differentiate into memory and effector cells that express skin-homing receptors like CLA and chemokine GCPRs CCR4 and 10. Chemokines CCL17 and 22 are produced by keratinocytes (among other cells). The effector T cells enter the blood and can enter the skin via skin-specific adhesion molecules and chemokines. When an allergen reenters the skin Th2 cells elicit a immune response via cytokine release and IgE antibody response causing chronic skin inflammation and localized tissue destruction. Chronic inflammatory reaction sustained by lymphocytes, eosinophils and other inflamm cells that are attracted out of the blood vessels and migrate through the dermis using a gradient of chemokines bound to the ECM. T cells at the area can also produce chemokines to cause other T cells to upregulate their adhesion molecules and increases recruitment of T cells into the affected tissue. TNFa and IFNg lead to increased blood flow, increased vascular permeability, and increased immigration of leukocytes, fluid, and protein into the area.

Lesions contain mononuclear cell infiltrate predominantly located in the dermis and is composed of activated memory CD4 T cells (principally CLA CCR4 Th2 cells) and macrophages. IL4 and 13 cause keratinocytes and fibroblasts to secrete cytokines to attract skin-homing T cells, eosinophils, and macrophages into the skin. IL 4 and 13 also increase IgE synthesis. IL4 stimulates Th2 differentiation. IL5 promotes differentiation of eosinophils. IL 31 induces itching, Fas L and TNFa induce keratinocyte damage. Also downregulattion of antimicrobial peptides by keratinocytes leading to increased susceptibility to cutaneous bacterial and viral infections such as herpes simplex type 1.

Langerhans cells and macrophages also have IgE bound to the surface through CD23 which presents Ag to naïve T cells and the bound IgE allows the APCs to concentrate the Ag and make them more efficient at Ag presentation. IgE is also bound to mast cells via FceRI. Cross linking leads to production and secretion of IL 4 and 5 by mast cells and further biases the Th2 response.

In chronic atopic dermatitis there is a mix of Th1 and Th2 cells. The dermis is also infiltrated by DCs that have IgE bound. After cross linking they decrete lots of IL12 and may be used in inducing T cells to secrete IFNg (hallmark of chronic atopic dermatitis lesions).

Many patients also have allergen induced asthma and food allergy because sensitization directly through the skin can occur and hyperresponsiveness can occur.

**Case:** 2 yr old boy, worsening eczema, many open skin lesions that ooze clear liquid, increased itching, redness, and swelling, many different allergic reactions when young (wheezing and hives), low weight and height, skin had large scales and pustules, thickened plaques of skin with deep criss cross pttern around creases of elbows and knees and backs of hands and feet, positive skin culture for staph and strep (treat with antibiotics), high eosinophil count, high serum IgE, increase serum tryptase levels 4 hrs. after milk challenge (shows IgE induced mast cell degranulation), herpes simplex infection (treated with acyclovir antiviral)

**Treatment:** emollients (coal tar, softening the underlying dry skin and reducing inflammation, improve skins barrier function), avoidance of irritants, immunosuppressants such as cyclosporin A (acts on T cells to interfere with cytokine gene transcription by binding to cyclophilin and inhibits calcineurin which dephosphorylates NFAT (cytokine gene TF), ALSO FK506 by binding to its BP and inhibiting calcineurin), acute flare up treatment with topical corticosteroids and or calcineurin inhibitors, antihistamines to control itching.

**Recognize that atopic dermatitis (AD) is associated with increased IgE and impaired T cell-mediated immunity, especially in the skin, and that there is a strong association of the disease with a personal or family history of atopy.**

**Explain that cells in AD skin lesions are predominately CD4 cells and macrophages, and that these CD4 cells produce IL-4, IL-5 and IL-13 (Th2-type) cytokines that inhibit Th1 responses.**

**Explain that T cells entering the skin express CLA, which allows the cells to bind to E-selectin and subsequently migrate out into the skin.**

**Recognize that immunosuppressive drugs can be used to treat AD, which appear to mediate their effects by reducing the production of inflammatory cytokines.**

**Recognize that extensive herpesvirus infection is often experienced by AD patients, which is believed due to the inhibition of Th1 responses in the skin.**

Defective local innate cell mediate immunity, Th2 cytokine expression in the skin inhibitts production of antimicrobial peptides by keratinocytes. Selective activation of Th2 cells so cell mediated responses via Th1 and Tc cells is reduced. Monocytes also secrete increased amounts of IL10 and prostaglandin E2 wich inhibit IFNg production. IL10 inhibits T cell mediated reactions also.

**Recognize that AD skin is usually colonized by *Staphylococcus aureus* and Group A streptococci.**

Can maintain skin inflammation by secreting superantigens which cause polyclonal stimulation of T cells and macrophages. Increased IL5 production, CLA receptor in T cells (skin homing, mediated by IL2). Produce IgE directed against staph superantigens and release IgE histamine upon exposure.

**Explain both the importance of allergen avoidance in preventing flare-up of AD and the appropriate use of immunosuppressive drugs for treatment of AD.**

**Corticosteroid use?**

Bind to steroid receptors on T cells and eosinophils and complex is translocated into the nucleus to increase synthesis of the inhibitor of NFkB (which controls expression of multiple cytokine genes). Inhibits cytokine cynthesis and the release of preformed mediators. Overuse can lead to local skin atrophy

***Case 53: Contact Sensitivity to Poison Ivy: Hypersensitivity reaction Type IV***

**Summary:** Most frequently encountered delayed hypersensitivity reaction in the US (allergic contact dermatitis). T cell response to pentadecacatechol (small highly reactive lipid-like molecule that penetrates the outer layers of the skin and binds covalently and non specificallty proteins on the surfaces of skin cells and functions as a hapten). Most people are susceptible and sensitivity is lifelong. Each subsequent exposure to Ag leads to decreased period of latency from contact to appearance of rash (anamnesis). Each reexposure also generates more effector and memory T cells that can persist a lifetime. Delay due to time it takes to recruit Ag specific T cells and other cells to the site. No fever or malaise. Occurs 2-3 days after exposure. Raised, red, weeping blisters. usually presents w/ a linear pattern of blisters called Hoebner phenomenon. Skin lesions are due to heavy infiltration of the contact sites w/ blood cells combined w/ localized destruction of skin cells. Rash only occurs in areas of initial contact (or where someone touched before they washed off).

Afferent response is when hapten enters the epidermis and haptenated self-proteins are phagocytized by langerhans cells in the epidermis and DCs in the dermis. Cleaved into peptides in intracellular vesicles and presented on the surface via MHCII. Over the next 12-48 hrs. the APCs travel to a node where they present Ag to activate naïve hapten specific T cells to become effector Th1 cells and CD8 cells that express skin homing receptors like E selectin and CCR4. Efferent response when the Th1 and CD8 cells go to the site of contact with the plant and react with haptenated peptides presented by the APCs leading to release of inflammatory mediators by Th1 cells and cytotoxic molecules by Tc cells.

Th1 mediators include IFNg to activate macrophages leading to their release of cytokines, chemokines (CCL5) and inflammatory mediators such as ILs, prostaglandins, NO, and LTs. IL3 and GMCSF can induce monocyte produce in the bone marrow. TNFa increases expression of adhesion molecules on endothelium lining postcapillary venules and increases vascular permeability so macrophages and other leukocytes can adhere to BVs and migrate to tissues in response to chemokines. Once at the site macrophages are activated and release cytokines and other mediators to attract more monocytes, T cells and leukocytes to amplify and maintain the reaction. BVs dilate (redness) and mast cells degranulate and release histamine (itching). Tissue destruction caused by cytokines and cell interactions. TNFa and lymphotoxin release by Th1 cells and macrophages act on TNF receptors expressed on skin cells to stimulate apoptosis. Also use FasL. Stromelysin can also degrade proteins of extracellular matrix that maintain skin integrity.

Haptens can also diffuse through the plasma membrane to the cytosol and bind to intracellular proteins. Peptides can be presented via MHCI and activate CD8 cells that attack tissue cells that present the Ag on their surface after they have been primed.

**Case:** itchy red skin eruptions 2 days after a hike, antihistamine decreased the itching, rash remains week after first appearance, large patches of raised, red, elongated blisters oozing clear fluid, swollen body parts, no fever, fatigue, or other symptoms

**Treatment:** corticosteroids (inhibit cytokine and chemokine production by diffusing across the PM and complexing to receptor proteins in the cytoplasm, enter nucleus and control gene expression including production of inhibitor of TFs, oral for more severe cases to achieve concentration necessary to inhibit the inflammatory response), antihistamines to block hisamine receptors and counteract itching

Can have reoccurance of symptoms as long as haptenated peptides are being generated

Pt with x linked agammaglobulinema still at risk because Ab doesn’t play a role in delayed hypersensitivity rxns

Use delayed hypersensitivity rxns for TB test. Small amounts of tuberculin injected and a day or 2 later a sensitized person develops a small red raised area at the site of injection.

**Explain how tissue damage can be produced as a result of a type IV (delayed-type) hypersensitivity reaction, and in particular distinguish damage caused by Th1 type effectors from damage produced by cytotoxic T cells (CTLs).**

**Explain that contact sensitivity results from a mixture of both Th1 and CTL responses in a sensitized person.**

**Explain that sensitization for contact sensitivity follows binding to self proteins by haptens, and that presentation of these haptenated peptides can then occur via MHC class II molecules, leading to inflammatory responses that includes the production of cytokines, such as TNF and IFN-.**

**Describe the process by which haptens penetrating the cell cytosol (such as lipidated haptens) may react with intracellular proteins, and haptenated peptides may end up being expressed on MHC class I molecules that provide cell targets for CTLs.**

**Explain that the most common cause of contact sensitivity in the USA is poison ivy, but that contact sensitivity can result from contact with many materials, including nickel ion.**

Insect bites/stings, beryllium, gliadin (celiacs disease)

**Explain that, on first exposure to a sensitizer, it can take a week or more to develop a lesion, and this occurs only if enough sensitizer remains in the skin. A second exposure in an immune person can lead to rash development within 24-48 h.**

**Explain that typical treatment for contact sensitivity includes use of anti-inflammatory steroids, in addition to attempting to remove the sensitizer with detergents, and advice to avoid those sensitizers in the future.**

**Explain how contact sensitization can be determined using patch tests and what a patch test entails**

Confirm Ag via patch test where material with hapten is applied to skin for 48 hrs and then checked. Could also incubate peripheral blood mononuclear cells with hapten and check T cell proliferation 6-9 days later.